

Anabolic Steroids In Urine For GC Or GC/MS Confirmations Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

ZSDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
Without Tips

Or

ZCDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
With CLEAN-THRU® Tips

Procedure:

1. Prepare Sample

- To 5 mL of urine add internal standard(s)* and 2 mL of β -Glucuronidase.
- β -Glucuronidase: 5,000 F units/mL *Patella vulgata* in 100 mM acetate buffer (pH 5.0).
- Mix/vortex.
- Hydrolyze for 3 hours at 65°C. Cool before proceeding.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Adjust sample pH to 6.0 ± 0.5 with approximately 700 μ L of 1.0 N NaOH.

2. Prepare Clean Screen® Extraction Column

- 1 x 3 mL CH_3OH .
 - 1 x 3 mL D.I. H_2O .
 - 1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
- Note:** Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

- Load at 1 to 2 mL/minute.

4. Wash Column

- 1 x 3 mL 10% (v/v) CH_3OH in D.I. H_2O .
 - Dry column (5 minutes at > 10 inches Hg).
 - 1 x 1 mL hexane or hexane/ethyl acetate (50:50).
- ** Part # SMSTFA-0-1,10, 25, 100

5. Elute Anabolic Steroids (Choose a, b, c or d)

- 1 x 3 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78:20:2); Collect eluate at 1 to 2 mL/minute.
- Note:** Prepare elution solvent daily. Add IPA/ NH_4OH , mix, then add CH_2Cl_2 (pH 11-12).
- 1 x 3 mL $\text{CH}_2\text{Cl}_2/\text{IPA}$ (80:20).
 - 1 x 3 mL ethyl acetate.
 - 1 x 3 mL CH_3OH .

6. Dry Eluate

- Evaporate to dryness at < 40°C.

7. Derivatize

- Add 50 μ L ethyl acetate and 50 μ L MSTFA** (with 3% trimethylsilyliodide).
 - Over layer with N_2 and cap.
 - Mix/vortex.
 - React 20 minutes at 70°C.
 - Remove from heat source to cool.
- Note:** Do not evaporate MSTFA solution.

8. Quantitate

- Inject 1 to 2 μ L onto chromatograph.
- Monitor the following ions (GC/MS):



Compound	Primary*	Secondary	Tertiary	Other
Testosterone-TMS	432	301	209	
19-Noretiocholanone-TMS	405	315	225	
Oxymethalone	640	52	462	370,143
Dehydroepiandrosterone-2TMS	432	327	297	
10-Nortestosterone-2TMS	418	287	194	
Oxymethalone Metabolite #1	640	52	462	143
Oxymethalone Metabolite #2	625	462	370	143
11-β-Hydroxyandosterone	522	417	158	
Methandienone	409	313	281	
19-Norandosterone-2TMS	405	315	225	
Alpha-Hydroxyetiocholanone	504	417		
17-α-Epitestosterone-TMS	432	341	327	209
Stanozolol	472	381	342	149

*Quantitation Ion

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255

www.unitedchem.com Email: methods@unitedchem.com

©UCT, LLC 2008 • All rights reserved

